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Comparison of lantibiotic gene clusters and encoded proteins

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Abstract

Lantibiotics form a group of modified peptides with unique structures, containing post-translationally modified amino acids such as dehydrated and lanthionine residues. In the gram-positive bacteria that secrete these lantibiotics, the gene clusters flanking the structural genes for various linear (type A) lantibiotics have recently been characterized. The best studied representatives are those of nisin (*nis*), subtilin (*spa*), epidermin (*epi*), Pep5 (*pep*), cytolysin (*cyl*), lactocin S (*las*) and lacticin 481 (*lct*). Comparison of the lantibiotic gene clusters shows that they contain conserved genes that probably encode similar functions.

The *nis*, *spa*, *epi* and *pep* clusters contain *lanB* and *lanC* genes that are presumed to code for two types of enzymes that have been implicated in the modification reactions characteristic of all lantibiotics, i.e. dehydration and thio-ether ring formation. The *cyl*, *las* and *lct* gene clusters have no homologue of the *lanB* gene, but they do contain a much larger *lanM* gene that is the *lanC* gene homologue. Most lantibiotic gene clusters contain a *lanP* gene encoding a serine protease that is presumably involved in the proteolytic processing of the prelantibiotics. All clusters contain a *lanT* gene encoding an ABC transporter likely to be involved in the export of (precursors of) the lantibiotics. The *lanE*, *lanF* and *lanG* genes in the *nis*, *spa* and *epi* clusters encode another transport system that is possibly involved in self-protection. In the nisin and subtilin gene clusters two tandem genes, *lanR* and *lanK*, have been located that code for a two-component regulatory system.

Finally, non-homologous genes are found in some lantibiotic gene clusters. The *nisI* and *spaI* genes encode lipoproteins that are involved in immunity, the *pepI* gene encodes a membrane-located immunity protein, and *epiD* encodes an enzyme involved in a post-translational modification found only in the C-terminus of epidermin. Several genes of unknown function are also found in the *las* gene cluster.

A database has been assembled for all putative gene products of type A lantibiotic gene clusters. Database searches, multiple sequence alignment and secondary structure prediction have been used to identify conserved sequence segments in the LanB, LanC, LanE, LanF, LanG, LanK, LanM, LanP, LanR and LanT gene products that may be essential for structure and function. This database allows for a rapid screening of newly determined sequences in lantibiotic gene clusters.

Introduction

Lantibiotics constitute a unique class of antimicrobial peptides which are produced exclusively by gram-positive bacteria and are mainly effective against other gram-positive bacteria. Their unique structural properties result from the presence of intra-molecular rings formed by thioether bonds of lanthionine and 3-methyllanthionine residues (Jung, 1991). In addition, the dehydrated residues dehydroalanine (Dha) and dehydrobutyrine (Dhb) are commonly found in lantibiotics, and other modified residues including D-amino acids are occasionally found (Kupke et al., 1992; Skaugen et al., 1994). These unusual amino acids are produced by post-translational modification of ribosomally produced precursor peptides. Most of these modifications are assumed to occur intracellularly and to require specific enzymes. Maturation of the modified precursors is complete after cleavage of the leader peptide and translocation across the cell membrane.

In recent years it has become evident that the various gene products required for biosynthesis, processing and translocation of lantibiotics, but also those required for self-immunity are encoded in gene clus-

Table 1. Encoded proteins (and number of residues) of lantibiotic gene clusters.

lantibiotic	acronym[a]	organism	precursor	modification			protease	regulation		transport / immunity				immunity
			A	B	C/M	D	P	R	K	T	F	E	G	I
nisin	Nis	*Lactococcus lactis*	57	**993**	**414**		**682**	**229**	**447**	**600**	**225**	241	214	245
subtilin	Spa	*Bacillus subtilis*	56	**1030**	**441**			**220**	**387**	**614**	<-447->		203	165
epidermin	Epi	*Staphylococcus epidermidis*	52	**990**	**416**	181	**461**	205		[b]**501>**	**231**	254	230	
Pep5	Pep	*Staphylococcus epidermidis*	60	**967**	**398**		**285**			**576**				69
epilancin K7	Elk	*Staphylococcus epidermidis*	55				**130>**			**95>**				
cytolysin	Cyl	*Enterococcus faecalis*	68/63		**993**		**412**			**714**				
lactocin S[c]	Las	*Lactobacillus sake*	68		**925**		**266**			**535**				
lacticin 481	Lct	*Lactococcus lactis*	51		**922**					**691**				
salivaricin	Sal	*Streptococcus salivarius*	51											
streptococcin	Scn	*Streptococcus pyogenes*	51											

[a]: Nomenclature in original references is indicated in brackets: NisA (SpaN), SpaA (SpaS), SpaB (SpaD/SpaE), SpaT (SpaY), EpiR (EpiQ), EpiT (EpiY), CylA1 (CylL$_L$), CylA2 (CylL$_S$), CylP (CylA), CylT (CylB), LctA (LcnDR1), LctM (LcnDR2), LctT (LcnDR3).
[b]: *epiT* gene is interrupted and incomplete: EpiT' has 148 residues, while EpiT'' has 353 residues and is incomplete at the C-terminus.
[c]: Five other proteins (239, 93, 125, 57 and 414 residues) of unknown function are encoded in the lactocin S cluster.
Bold: *homologous family*

Literature references and accession codes:
Nisin: Buchman et al., 1988 (J04057); Kaletta and Entian, 1989 (M24527); Dodd et al., 1990 (M27277); Mulders et al., 1991 (X61144); Steen et al., 1991 (M65089); Engelke et al., 1992 (X68307); Van der Meer et al., 1993 (L11061); Kuipers et al., 1993a (L16226); Engelke et al., 1994 (X76884); Siegers and Entian, 1995 (U17255); Immonen et al., 1995 (Z18947, Z22725, Z22813).
Subtilin: Banerjee and Hansen, 1988 (J03767); Chung et al., 1992 (M83944); Chung and Hansen, 1992 (M99263); Klein et al., 1992, 1993 (L07785); Gutowski-Eckel et al., 1994 (L24075); Klein and Entian, 1994 (U09819).
Epidermin: Schnell et al., 1988, 1992 (X62386); A.Peschel and F.Götz, submitted (EpiEFG). M. Otto and F. Götz, personal communication (EpiT). Gallidermin: Schnell et al., 1989 (A61072).
Pep5: Kaletta et al., 1989 (JL0100); Reis et al., 1994 (L23967); Meyer et al., 1995 (Z49865).
Epilancin K7: Van der Kamp et al., 1995 (U20348).
Cytolysin: Gilmore et al., 1990 (M38052); Segarra et al., 1991 (M38052); Segarra, 1992; Gilmore et al., 1994 (L37110).
Lacticin 481: Piard et al., 1993 (X71410); Rince et al., 1994 (Lactococcin DR; U04057).
Lactocin S: Skaugen et al., 1994; Skaugen, 1994; M.Skaugen and I.Nes, unpublished results.
Salivaricin: Ross et al., 1993 (L07740).
Streptococcin: Hynes et al., 1994a (L11653), 1994b (L36235).

ters. Many of these gene products show significant amino acid sequence homology and hence are assumed to perform similar functions. In this review we will summarize the recent progress made in the identification and comparison of these genes and their encoded proteins. We focus upon the linear (type A) lantibiotics since presently very little genetic information is available for the circular (type B) lantibiotics.

Organization of gene clusters

Gene clusters flanking the structural genes for the lantibiotics produced by various gram-positive bacteria, listed in Table 1, have been characterized and their organization is summarized schematically in Figure 1. To facilitate comparison, a common nomenclature for the genes is used as proposed previously (de Vos et al., 1991). Two classes of gene clusters can be distinguished: the best characterized genes of the first class are those of nisin, subtilin, epidermin and Pep5, and those of the other class are cytolysin, lacticin 481 and lactocin S (Fig. 1).

In addition to the structural genes encoding the lantibiotic prepeptides (LanA), other homologous genes are found encoding proteins involved in regulation of gene expression (LanR, LanK), transport (LanT), self protection (LanFEG) and proteolysis (LanP). The *nis*, *spa*, *epi* and *pep* clusters contain homologous *B* and *C* genes that are presumed to code for two types of enzymes that have been implicated in the modification reactions characteristic of all lantibiotics. The *cyl*, *las* and *lct* gene clusters have no homologue of the *B* gene, but they do contain a much larger *M* gene that is the *C* gene homologue.

Non-homologous genes are found in some lantibiotic gene clusters. The *nisI* and *spaI* genes encode

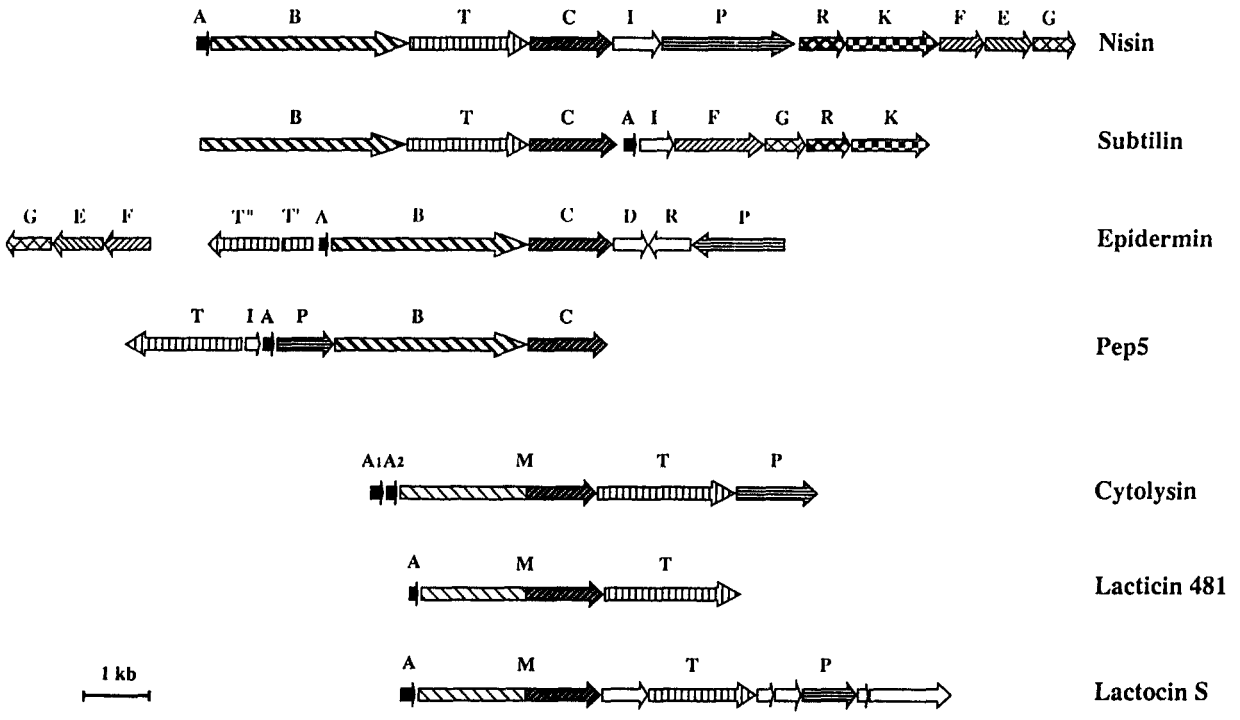

Fig. 1. Organization of lantibiotic gene clusters. The structural *lanA* genes are shown in black. Genes encoding similar functions, as deduced from sequence homology, have identical shading patterns; genes encoding non-homologous proteins are not shaded. Nomenclature is standardized according to De Vos et al. (1991). Nomenclature in original references, when different, follows in brackets: *spaA (spaS), spaB (spaD/spaE), spaT (spaY), epiR (epiQ), epiT (epiY), cylA1 (cylL$_L$), cylA2 (cylL$_S$), cylP (cylA), cylT (cylB), lctA (lcnDR1), lctM (lcnDR2), lctT (lcnDR3).*

lipoproteins that are involved in immunity (Kuipers et al., 1993; Klein and Entian, 1994), the *pepI* gene encodes a membrane-located immunity protein (Reis and Sahl, 1991), and *epiD* encodes a gene product involved in a post-translational modification only found in the C-terminus of epidermin (Kupke et al., 1992). Several genes of unknown function are also found in the *las* gene cluster.

Apparently there is no uniform order or orientation of the genes in the different gene clusters, as rearrangements have occurred during evolution. The organization of the *nis* and *spa* gene clusters is most conserved which reflects the high structural similarity between the two lantibiotics nisin and subtilin. Some homologous genes are absent in individual gene clusters, suggesting either that (a) such genes are located elsewhere, (b) such genes are not required at all, or (c) sequencing of the gene cluster is incomplete. For instance, it is quite likely that other genes will be found by sequencing downstream of *lctT* (Figure 1).

Identification and comparison of gene products

The function of genes and their translation products can and should be established experimentally, for instance by gene transfer, mutagenesis, inactivation or overproduction, or by the isolation and characterization of gene products. Many of such studies have been performed for genes in lantibiotic gene clusters, and they are reviewed elsewhere (Sahl et al., 1995; Jack et al., 1995; Jack and Sahl, 1995).

In the present review we focus primarily on the identification and comparison of gene products using computer analysis methods, such as database searches, multiple sequence alignment, secondary structure and helical transmembrane prediction (Rost and Sander, 1994), hydropathy analysis (Kyte and Doolittle, 1982) and molecular modelling by homology.

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NisB        (64) -LKKKRVKK- (14) -RSTPFGLFS- (242) -YKDKFIEKYG- (254) -PRLVFDEIVISPAKWKI-
SpaB        (67) -LKGKKKRN- (14) -RTTPFGLFS- (244) -YHNVFLERYG- (260) -PEIKYKEITLSCEQWLI-
EpiB        (64) -KDNKKTRN- (14) -RSTPYGMLS- (247) -YHEFFMDKYG- (246) -PRIIYKNIILKPATWKI-
PepB        (67) -LSKKKKKN- (14) -RSTPFGFMS- (247) -YKNKFLEIYG- (229) -PRIVYKNIIISPRKWKI-

consensus          L..Kk..n        RsTPfG..S         Y...F.e.YG         Pri.yk.I..sp..WkI


                                                                                 total residues
NisB      - (153) -FFLRYTDPKPHIRLR- (33) -YDQEVERYGG- (128) -SIIHVHNNRL- (26)        993
SpaB      - (163) -FFMRYRDPKPHIRLR- (35) -YEREIERYGG- (136) -SIIHLHCNRL- (34)       1030
EpiB      - (145) -FYIKFKEDEDFIKLR- (35) -YVPEVYRYGG- (127) -SFIHMRCNRI- (29)        990
PepB      - (150) -YFIRYADEKEHIRLR- (31) -YYRETYRYGG- (121) -SIVHMFNNRL- (29)        967

consensus          ff.ry.d.k.hIrLR        Y..E..RYGG         SiiH...NRl
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Fig. 2. Strongest homology regions of putative modification enzymes "LanB" for dehydration and/or thioether formation. In brackets are the number of residues between conserved regions. Consensus: fulled conserved residues in upper case, highly conserved residues in lower case.

Lantibiotic precursor (LanA)

The structural genes of various lantibiotics have been characterized in recent years and were used to deduce the leader and pro-lantibiotic sequences. The primary translation products range in size from 51–68 residues and the pro-peptides from 22–37 residues. Remarkably, there are tandem structural genes *cylA1* and *cylA2* for the production of cytolysin and both encoded peptides are necessary for activity (Gilmore et al., 1994). Despite the fact that lantibiotics all have thioether rings and dehydrated residues, there is no overall homology pattern in the LanA peptides. NisA and SpaA (59% identical residues) are the most homologous, followed by the set LctA, SalA and ScnA (32–55%), and PepA and ElkA (35%); LasA and CylA1 show weak homology (24%).

Sequence comparison of mature lantibiotics, leader peptides and natural variants is described in more detail elsewhere (Sahl et al., 1995; De Vos et al., 1995).

Modification (LanB, LanC, LanM)

Two categories of enzymes have been implicated in the modification reactions characteristic of all lantibiotics, i.e. the dehydration of serine and threonine residues in the propeptide region and subsequent thioether ring formation. One class includes the LanB proteins encoded in the *nis, spa, epi* and *pep* gene clusters, while the other comprises the LanC proteins encoded in the same clusters and their LanM homologues in the *cyl, lct* and *las* gene clusters. Although these proteins show sequence similarity with each other (Figs. 2 and 3), no other sequences were found in protein or nucleotide databases that had a significant resemblance to any of the putative modifying enzymes.

The LanB proteins all consist of about 1000 residues. Their overall sequence similarity is not high (26–29% identical residues), but there are seven short segments of stronger homology that may have functional significance (Fig. 2). The most conserved segment is near the C-terminus and has a consensus YxxExxRYGG sequence. However, it is impossible to predict which residues are essential for catalysis or other functions since over 75 residues are identical in all four LanB proteins. These proteins are predominantly hydrophilic and have a high content of predicted secondary structure (Gutowski-Eckel et al., 1994), predominantly helical, and few hydrophobic regions (R.J.Siezen, unpublished results). It has been demonstrated that the *lanB* gene products are essential for lantibiotic production (Klein *et al.*, 1992; Augustin *et al.*, 1992; Chung and Hansen, 1992; Kuipers *et al.*, manuscript in preparation). LanB has been proposed to play a role in dehydration since it has weak homology to IlvA, a threonine dehydratase from *E.coli* (Gutowski-Eckel et al., 1994). However, the similarity regions with IlvA do not correspond to the conserved LanB segments in Fig. 2.

The homologous LanC proteins encoded in the *nis, spa, epi* and *pep* clusters are all about 400 residues long (Fig. 4). NisC and SpaC are most similar (33% sequence identity) while similarity between the others varies from 24–32% identity. Hydropathy analy-

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N-terminal domain
LctM       (223)-YYRKIGVLLS VAYTLNLTDL HFENVISQGE NPCIIDLETM-(659)
CylM       (328)-YYERYGKLIG IAFLFNVTDL HYENIIAHGE YPVIIDNETF-(625)
LasM       (236)-YYYRSGCLLG LFWILGTTDI HSENIITNAG YPIVIDIETL-(649)

consensus        YY.r.G.Llg .a..ln.TDl H.ENiI..ge yP.iID.ET.


C-terminal domain
NisC         (34) -LSTGLPGII- (38) -SLYSGAAGIAL- (44) -DVIEGLSGIL- (58) -GLAHGLAG-
SpaC         (52) -LSHGIPGIC- (38) -SMFSGAAGIGL- (44) -DVIEGVSGIA- (63) -GLAHGIPG-
EpiC         (51) -LSHGYPGII- (36) -SLFSGLSGIGF- (43) -DIIQGFSGIG- (57) -GLAHGILG-
PepC         (35) -LITGYPGIA- (38) -SLFEGAFGTIF- (48) -DIISGCAGTL- (49) -GYAHGIPG-
LctM        (580) -IYKGISGIG- (40) -GFYVGLIGEYS- (37) -DVIAGEAGII- (34) -SYAHGNSG-
CylM        (693) -MYDGLPGIF- (35) -SAFFGKGSLIY- (38) -DWIHGHNSII- (35) -GFGHGIYS-
LasM                          (635) -SVFSGLGGLLY- (39) -EYLNGISGLL- (41) -GIGHGISG-

consensus          l..G.pGI.         s.fsG..g...         d.i.G.sgi.         g.aHGi.g
                                                                             *

                                                                        total residues
NisC       - (66) -WCYGGPGI- (39) -CHGYSGL- (40) -GFLEGISGC- (33)          414
SpaC       - (66) -WCYGRPGV- (39) -CHGYSGI- (56) -GLLDGAVGV- (21)          428
EpiC       - (59) -WCYGDTGI- (40) -CHGLASH- (55) -GILEGELGV- (25)          441
PepC       - (55) -WCYGLPSV- (42) -CHGFSGV- (45) -GLLNGNAGI- (24)          398
LctM       - (50) -WCHGASGQ- (48) -CHGILGN- (49) -GLMTGISGI- (22)          922
CylM       - (34) -WCKGTVGE- (29) -CHGNAGT- (47) -GFFVGISGV- (20)          993
LasM       - (40) -WCRGTLGI- (43) -CHGLGGI- (42) -SYMLSTTGL- (23)          925

consensus          WCyG..g.          CHG..g.          g.l.g..G
                   **                **
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Fig. 3. Strongest homology regions of putative modification enzymes "LanC" and "LanM" for dehydration and/or thioether formation. * , putative essential residues. Other details as in Fig.2.

sis has identified a rather regular alternation of eight hydrophilic and seven hydrophobic regions in these LanC proteins (Engelke et al., 1992). The seven hydrophobic segments show the strongest sequence conservation, but they are not typical transmembrane sequences (Fig. 3); the eight hydrophilic segments are all predicted to contain a long amphipathic helix as secondary structure (R.Siezen, unpublished results). These structural characteristics point towards a globular structure with alternating α-helices and ß-sheet strands.

Remarkably, the LanM proteins of over 900 residues encoded in the *cyl, las* and *lct* gene clusters contain a C-terminal domain with clear homology to the LanC proteins, i.e. 20-27% sequence identity. The same seven strongly conserved sequence segments found in LanC are also found in LanM proteins (Fig.3). It is postulated that both the LanC proteins and their LanM homologoues are involved in the formation of thioether bonds from the dehydrated residues and cysteines; recently, experimental support for this role has been obtained for PepC (Meyer et al., 1995).

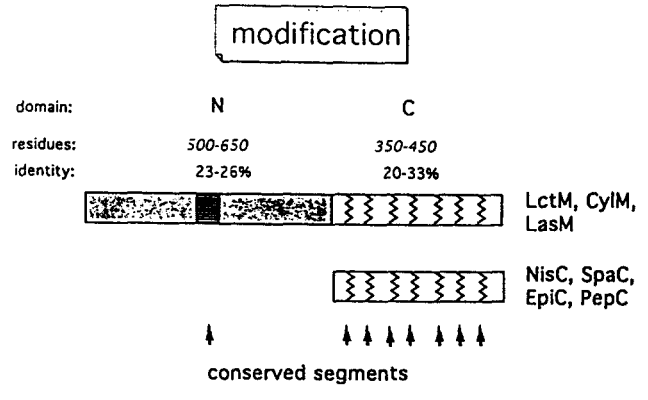

Fig. 4. Schematic representation of putative modification enzymes "LanC" and "LanM". Domain size, number of residues, overall sequence identity (%) and position of strongest homology regions are indicated. Homologous domains have identical shading patterns. N, amino-terminal domain; C, carboxy-terminal domain.

The many conserved glycine residues in the consensus sequences of LanC/LanM (Fig.3) presumably play a role in structural conservation, while the only other

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NisP     (254) -VGIIDSGIMEEHPDL- (32) -DKMGHGTEVAGQI- (43) -KVINISAGQ-
Sub      (134) -VAVIDSGIDSSHPDL- (17) -DNNSHGTHVAGTV- (47) -DVINMSLGG-
EpiP     (145) -VALVDSGVNSSHTDL- (30) -DKLNHGTLVAGQI- (43) -DVINVSLGN-
PepP     ( 15) -ILFIDSGCDFKHPEL- (18) -DYTGHGTQIISVL- (44) -KVINISFSG-
CylP     (137) -IALIDSGIDRLHPNL- (16) -DEYGHGTQVAGVI- (37) -DIINVSLGS-
LasP     ( 28) -IALLDENINTTHSYL- (12) -TASIHGTAMAGVL- (39) -KLIIFPMSI-

consensus       .a.iDsgi...Hp.L         d...HGT.vag..         .vIn.s.g.
                     *                       *


                                                                  residues
NisP   - (31) -GSIVVAALGND- (101) -GNSFATPKVSGALAL- (158)          682
Sub    - (17) -GVVVAAAAGNE- ( 62) -GTSMASPHVAGAAAL- ( 42)          382
EpiP   - (33) -GSIVVAAVGND- (101) -GTSLAAPKVSGALAL- ( 47)          461
PepP   - (21) -NIVICWSSMNN- ( 79) -GNSIATSYASGCFML- ( 45)          285
CylP   - (26) -NILIVASAGNE- ( 92) -GTSLATPEVSAALAA- ( 41)          412
LasP   - (26) -NATILSSAGND- ( 74) -GTSLATGLFAGYALT  ( 15)          266

consensus       .....a..gN.           GtS.Atp.vsga.al
                        *                   *
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Fig. 5. Strongest homology regions of putative peptidase "LanP" for leader peptide cleavage. Sub = subtilisin from *Bacillus subtilis*; *, catalytic residues. Other details as in Fig.2.

fully conserved residues (2 His, 2 Cys, 1 Trp) could be involved in catalysis, disulfide bond formation or metal-ion binding.

The N-terminal domains of over 500 residues of CylM, LctM and LasM have weak but significant overall homology (23–26% sequence identity), suggesting a similar function. The most conserved segment in the middle of this N-terminal domain has 50% mutual sequence identity and many fully conserved residues (Figs.3,4). Database searches with only the consensus sequences from LanB, LanC or LanM proteins did not identify any other proteins than those encoded in lantibiotic gene clusters. As there are no typical *lanB* genes in the *cyl, lct* and *las* gene clusters, it is possible that the large LanM proteins are hybrids that combine the role of dehydratase and lanthionine-forming capacity in two connected domains. However, since the N-terminal domains of the LanM hybrids do not show any significant sequence similarity to LanB proteins, the possibility that *lanM* genes have arisen during evolution through a fusion of ancestral *lanB* and *lanC* genes can be excluded.

A unique feature of the epidermin gene cluster is the presence of a gene, *epiD*, that encodes a flavoprotein involved in the formation of the unusual *S*-[(*Z*)-2-aminovinyl]-D-cysteine structure in epidermin (Kupke *et al.*, 1992). No homologues of *epiD* are present in the other gene clusters.

Protease (LanP)

The leader peptide is involved in maintaining the lantibiotic in an inactive conformation prior to translocation (van der Meer *et al.*, 1993; Kuipers *et al.*, 1993b; van der Meer *et al.*, 1994). Most gene clusters contain *lanP* genes encoding subtilisin-like proteases that are presumably involved in the proteolytic processing of the prelantibiotics. The most conserved regions of this serine protease family are around the active site residues Asp, His and Ser as well as the oxyanion-hole residue Asn of their catalytic domains (Fig.5)(Siezen et al., 1991). The highest overall homology is between NisP and EpiP (42% identical residues in the catalytic domain), and between PepP and ElkP (47% identity over the incomplete sequence of ElkP). Homology modelling has been used to predict the 3-dimensional structure of the NisP catalytic domain and its interactions with the nisin precursor (Siezen et al., 1995a).

The location of processing by a leader peptidase may differ depending on the lantibiotic (Fig.6). NisP has an N-terminal signal sequence and a C-terminal membrane anchor, indicative of secretion followed by anchoring to the cell membrane (Van der Meer et al., 1993). EpiP and CylP also contain a signal sequence but lack an anchor, suggesting that they operate extracellularly, either attached to the cell wall in another fashion or not attached at all (Segarra et al., 1991; Schnell et al., 1992); this extracellular location has

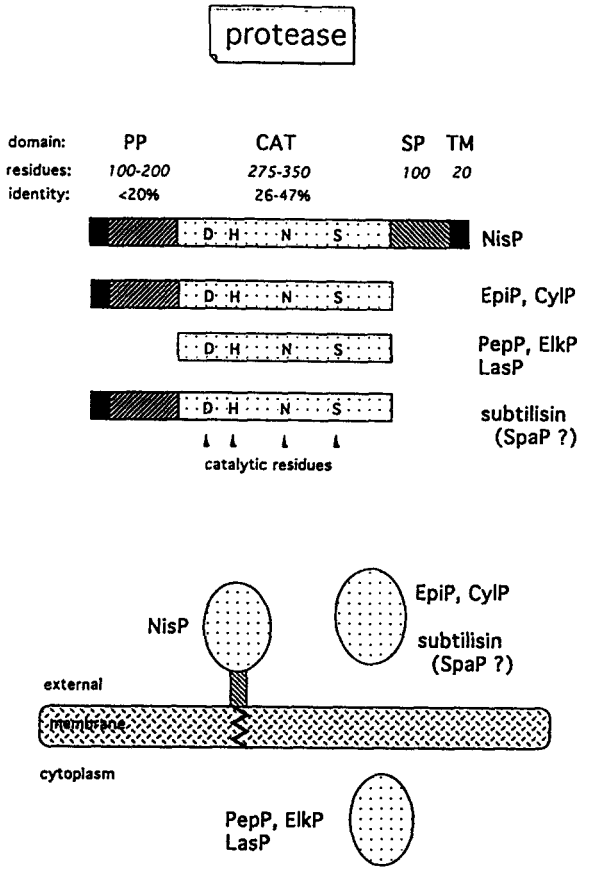

Fig. 6. Schematic representation of putative leader peptidases "LanP" and their predicted location. PP, prepro-peptide; CAT, catalytic domain; SP, spacer; TM, trans-membrane. Other details as in Fig.4.

recently been confirmed for EpiP (S.Geissler, F. Götz and T. Kupke, personal communication). The putative peptidases PepP, ElkP and LasP may function intracellularly, possibly as part of a large membrane-bound biosynthetic complex, since they lack a signal sequence and also have no pro-sequence which normally functions as an intramolecular chaperone (Shinde and Inouye, 1993; Siezen et al., 1995b). Although it cannot be excluded that other extracellular serine proteases substitute for their function, PepP at least appears to be essential (Meyer et al., 1995). The *spa* gene cluster does not contain a peptidase-like gene (Fig.1); however, the subtilin-producer *B. subtilis* is known to contain a variety of secreted serine proteases (e.g. subtilisin) that could be involved in the proteolytic activation of the lantibiotic subtilin.

Two classes of leader peptides have been distinguished in lantibiotics (De Vos et al., 1995). One class apparently needs the subtilisin-like protease LanP for cleavage. In the other class, the leader peptides are cleaved after Gly-Gly, Gly-Ala or Gly-Ser sequences such as found in the lantibiotic precursors for LctA, CylA1, CylA2, SalA and ScnA, but also in various non-lantibiotic bacteriocin precursors (Kolter and Moreno, 1992; De Vos et al., 1995). Such cleavage should require a different type of protease, since subtilisin-like proteases are not known to have this specificity. This other protease appears to be present as a domain of LanT (see below). Interestingly, both types of protease are encoded in the *cyl* gene cluster, and a two-step processing of the cytolysin leader peptide has been proposed (M.C.Booth and M.S.Gilmore, personal communication).

Transport (LanT)

ABC transporters are found to be encoded in all lantibiotic gene clusters (Fig. 1), and it is likely that these proteins are involved in the export of (precursors of) the lantibiotics; *sec*-dependent transport is unlikely since lantibiotics lack a typical signal sequence. Bacterial ABC exporters all contain an intracellular domain with the highly conserved ATP binding motif or cassette (ABC), also called Walker motif (Walker et al., 1982), indicating that ATP hydrolysis is required as a source of energy for secretion (Fath and Kolter, 1993). In addition, they have a membrane-spanning domain (MSD) that usually has six transmembrane helices. The MSD and ABC domains can either be on the same polypeptide (group A exporter), as in the well-characterized *E.coli* HlyB protein for export of hemolysin (Felmlee et al., 1985), or they can be separate proteins (group B exporters) as in the *E.coli* McbE (MSD) and McbF (ABC) proteins for export of microcin B17 (Garrido et al., 1988).

The LanT proteins all belong to the group A exporters, as they have an MSD with six predicted hydrophobic membrane-spanning segments coupled to a C-terminal domain with the conserved ATP-binding motif (Figs.7,8). The only exception is the *epiT* gene that is interrupted due to a frame shift, possibly leading to the synthesis of two separated domains. This transport role has been conclusively demonstrated for the export of cytolysin by CylT, exemplifying the first ABC exporter identified in gram-positive bacteria (Gilmore *et al.*, 1990).

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                                                                                    residues
NisT      (383) -IVGKNGSGKSTL- (99) -LSGGQWQKIALAR- (8) -YILDEPSAALD- (74)          600
SpaT      (384) -IVGPNGSGKKTF- (99) -LSGGQWQKIALAR- (8) -YILDEPSSALD- (87)          614
EpiT      ( ?) -IIGESGCGKSTL- (95) -LSIGQKQRLVLTR- (8) -LFY          ( ?)            ?
PepT      (367) -IVGPSGSGKSTI- (95) -LSGGQKQRINIAR- (8) -LLLDEATASLD- (70)          576
LctT      (494) -IVGKSGSGKSTL- (87) -LSGGQIQRLLIAK- (8) -IFWDEPFSSLD- (67)          691
CylT      (502) -IVGRSGSGKSTL- (96) -FSGGQRQKIALAR- (8) -LLLDEPTSAMD- (72)          714
LasT      (355) -LTGPNGSGKSML- (79) -LSTGQIQKIKFIR- (8) -LVLDEILENMD- (57)          535
HlyB      (499) -IVGRSGSGKSTL- (94) -LSGGQRQRIAIAR- (8) -LIFDEATSALD- (70)          707

consensus         ivG.sGsGKstl         lSgGQ.Qki.lar         l.lDEp...lD
                                  *                                  *

NisF      (31) -GLLGVNGAGKSTL- (79) -FSLGMKQRLGIGM- (8) -LILDEPTNGLD- ( 70)         225
SpaF      (34) -GLLGPNGAGKSTT- (82) -FSMGMRQRLGIAI- (8) -LILDEPTNGLD- (286)         447
EpiF      (34) -GLLGINGAGKSTL- (82) -FSLGMKQRLGIAM- (8) -LVLDEPSNGLD- ( 70)         231
McbF      (36) -GLLGENPAGKTTL- (83) -VSYGEKRWLIISL-(10) -FLLDEPTVGID- ( 80)         247

consensus        GLLG.NgAGKsTl         fS.GmkqrLgI..         l.LDEPtnGlD
                                  *                                  *
                  Walker A-site                          Walker B-site
```

Fig. 7. Strongest homology regions of ABC domain of translocators "LanT" and "LanF". HlyB, *Escherichia coli* ABC exporter for α-hemolysin (Felmlee et al., 1985); McbF, *Escherichia coli* ABC exporter for microcin B17 (Garrido et al., 1988). *, putative Mg^{2+}-ATP binding residues (Walker et al., 1982). Other details as in Fig.2.

In some cases the group A exporters have an extra N-terminal, hydrophilic intracellular domain of about 150 residues, as in HlyB from *E.coli* (Fath and Kolter, 1993) and in the ABC exporters for non-lantibiotic bacteriocins like pediocin from *Pediococcus* (Marugg et al., 1992), lactococcin A (Stoddard et al., 1992) and lactococcin G (L.Havarstein et al., 1995) from *Lactococcus*, plantaricin A from *Lactobacillus plantarum* (D.B.Diep, unpublished), colicin V from *E.coli* (Gilson et al., 1990) and competence factor from *Streptococcus pneumoniae* (Hui and Morrison, 1991). Such a homologous N-terminal domain with highly conserved residues is also present in both CylT and LctT (Figs.8,9). It has recently been shown that this extra domain represents a novel protease that is responsible for cleavage after Gly-Gly (or Gly-Ala, Gly-Ser) residues of leader peptides of both lantibiotic and non-lantibiotic peptide bacteriocins (L.Havarstein et al., 1995; Venema et al., 1995). The single Cys, His and Asp residues that are fully conserved (Fig. 9) in this domain may be involved in catalysis.

Regulation (LanR, LanK)

In the nisin and subtilin gene clusters two tandem genes, *nisRK* and *spaRK*, have been implicated in control of expression of their respective lantibiotic gene clusters (van der Meer *et al.*, 1993; Klein *et al.*, 1993; Engelke et al, 1994; Gutowski-Eckel *et al.*, 1994; de Vos *et al.*, 1994). The LanR and LanK proteins are homologous to a large family of two-component regulators (Stock et al., 1989; Albright et al., 1989; Parkinson, 1993; Swanson et al., 1994). One component of this system consists of a sensor that has two domains: the membrane-bound N-terminal domain receives an external signal and transduces that signal to its C-terminal cytoplasmic domain (histidine kinase) by autophosphorylating a His residue (Fig.11). Subsequently, the phosphate is transferred to an Asp residue in the N-terminal domain of an intracellular response regulator. This phosphorylation alters the activity of its C-terminal domain, which is presumably the transcriptional activator.

Homology analysis of NisR and SpaR (Fig.12) shows that the six most conserved segments with essential residues are present, as compared to *Bacillus* (Seki et al., 1987) and *E.coli* regulators (Comeau et al., 1985). Analysis of NisK and SpaK shows that both have two hydrophobic transmembrane regions in their N-terminal domain (Engelke et al., 1994), and conserved sequence segments with the essential His residue in the C-terminal domain. SpaK appears to be too short, lacking the last two conserved segments, but this may be due to a frame shift in the published nucleotide sequence (Klein et al., 1993) since anoth-

```
                                                                    residues
LctT      ( 5) -QNNEQDCLLA CYSMILGYFG- (64) -HFVVVTKIYR KNVTLIDP- (584)    691
CylT      ( 8) -QGEHSECALA CITMLLNYYG- (59) -HFVVIEKIKK KKVLILDP- (609)    714
CvaB      (25) -QTETAECGLA CLAMICGHFG- (59) -HFVVLVSVKR NRYVLHDP- (576)    698
LagD      ( 6) -QQDEKDCGVA CIAMILKHYG- (65) -HYVVVYKVKG DEIWIADP- (594)    703
LcnC      (10) -QVDEMDCGCA ALSMILKSYG- (65) -HYYVITGANK NSVFIADP- (603)    716
PedD      (12) -QVDENDCGLA ALNMILKYYG- (65) -HYYVVYQVTE NDLIIGDP- (609)    724
PlnG      ( 8) -QVDEMDCGVA ALAMILKNYG- (65) -HFYVVVKTSK THVVIADP- (605)    716
ComA      (10) -QVDQMDCGVA SLAMVFGYYG- (65) -HYYVVTGQDK DSIHIADP- (604)    717

consensus       Qvde.dCglA claMilkyyG       H..Vv.k..k ..v.iaDP
                          *                  *              *
```

Fig. 9. Strongest homology regions of N-terminal domains of translocators "LanT". LcnC, *Lactococcus* ABC exporter for lactococcin A (Stoddard et al., 1992); PedD, *Pediococcus* ABC exporter for pediocin PA-1 (Marugg et al., 1992); CvaB, *E.coli* ABC exporter for colicin V (Gilson et al., 1990); LagD, *Lactococcus* ABC transporter for lactococcin G (L.Havarstein et al., 1995); PlnG, *Lactobacillus* ABC transporter for plantaricin A (D.B.Diep, unpublished): ComA, *Streptococcus* ABC transporter for competence factor (Hui and Morrison, 1991). *, putative catalytic residues; other details as in Fig.2.

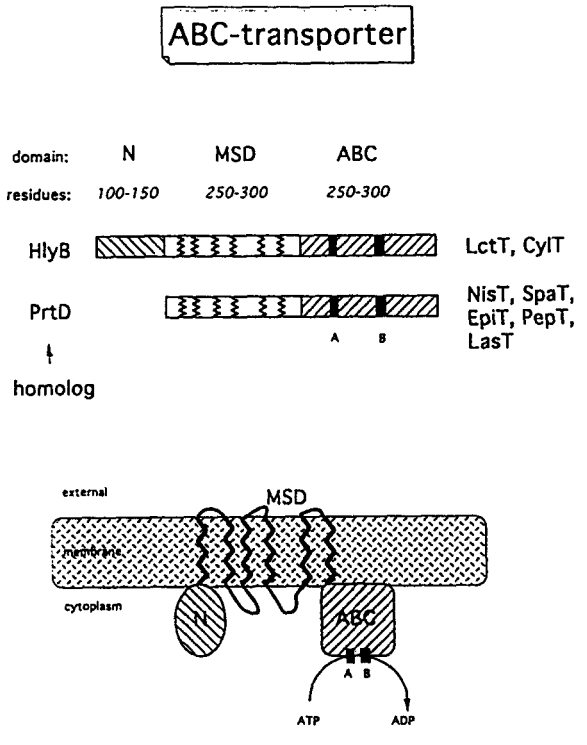

Fig. 8. Schematic representation of ABC transporters "LanT" and their predicted location with respect to the cell membrane. N, amino-terminal domain; MSD, membrane-spanning domain; ABC, ATP-binding cassette domain; black boxes A and B, Walker ATP-binding sites; zig-zag line, membrane-spanning segments. HlyB, *Escherichia coli* ABC exporter for α-hemolysin (Felmlee et al., 1985); PrtD, *Erwinia chrysanthemi* ABC exporter for protease D (Letoffe et al., 1990). Other details as in Fig.4.

er reading frame encodes at least another 27 residues with the 3rd conserved segment (italics in Fig.12). The external signal that is recognized by the sensor kinase LanK appears to be the lantibiotic itself, as has recently been demonstrated for nisin biosynthesis (Kuipers et al., 1995).

The *epiR* (or *epiQ*) gene codes for a protein with limited homology to NisR/SpaR that was shown to be a transcriptional activator of the *epi* gene cluster (Peschel *et al.*, 1993); no corresponding sensor kinase has been identified as yet.

Immunity or self protection (LanI; LanE, LanF, LanG)

The mechanism of immunity of lantibiotic-producing strains is still poorly understood. Genes encoding immunity proteins have only been identified in the gene clusters for Pep5 (Reis and Sahl, 1991), nisin (Kuipers et al., 1993) and subtilin (Klein and Entian, 1994) biosynthesis. The PepI protein consists of only 69 amino acid residues, with a striking 20 amino acid hydrophobic region between positions 6 and 27, suggesting a membrane-associated location of this protein. Localization studies confirmed this and demonstrated that PepI is located on the outer surface of the cytoplasmic membrane (Reis et al., 1994).

NisI and SpaI are both predominantly hydrophilic with a hydrophobic N-terminal signal sequence. These two immunity proteins have no sequence homology, but they both have a lipoprotein signal sequence with Cys at position +1 of the cleavage site for signal peptidase II (Kuipers et al., 1993; Klein and Entian, 1994). It

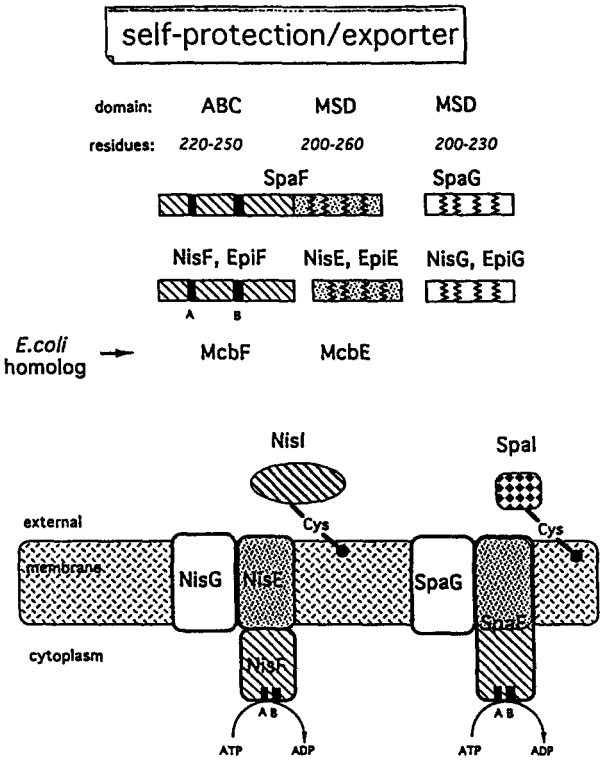

Fig. 10. Schematic representation of putative self-protection systems: immunity proteins "LanI" and ABC exporter proteins "LanE", "LanF" and "LanG" and their predicted location with respect to the cell membrane. N-terminal Cys residues of NisI and SpaI are attached to membrane lipids. McbE and McbF, *Escherichia coli* ABC exporter proteins for microcin B17 (Garrido et al., 1988). Other details as in Figs. 4 and 8.

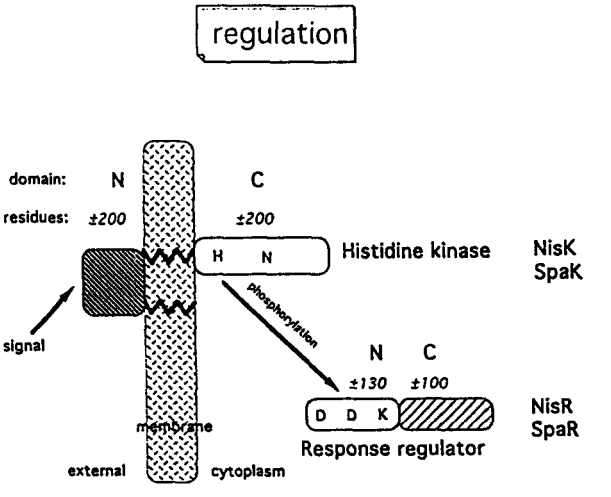

Fig. 11. Schematic representation of two-component regulator system: sensor kinase "LanK" and response regulator "LanR", and their predicted location in the cell. Essential conserved residues are indicated. Other details as in Figs. 4 and 8.

is assumed that these proteins become lipid-modified, extracellular membrane-anchored proteins after signal peptide cleavage (Von Heijne, 1989)(Fig.10).

A second ABC transporter system, belonging to the group B exporters, has recently been found in the *nis* (Siegers and Entian, 1995; T.Immonen and P.Saris, personal communication), *epi* (A.Peschel and F.Götz, personal communication) and *spa* (Klein and Entian, 1994) gene clusters, and this system has been postulated to participate in self-protection (immunity). The components of this transport system are now called LanE, LanF and LanG. NisF and EpiF are separate ABC domains homologous to *E.coli* McbF (Fig.7), while NisE, NisG, EpiE and EpiG are separate hydrophobic domains with predicted membrane-spanning segments resembling McbE (Fig.10). In SpaF one hydrophobic domain of 200 residues is still attached to the N-terminal ABC domain, while SpaG is a separate hydrophobic domain.

Conclusions and outlook

Based on the seven lantibiotic gene clusters that have been reasonably well characterized to date (Fig. 1) we can make some general conclusions about the genes and gene products required for biosynthesis and other functions. The primary translation product LanA (or LanZ: Mulders et al., 1991) is first modified enzymatically to generate dehydrated residues and lanthionines, that are exclusively located in the pro-peptide part, but never in the leader peptide. LanB, LanC and LanM are the obvious candidates for this enzymatic modification, since each gene cluster always encodes either LanM or the LanB/LanC combination. It is now clear that the C-terminal domain of LanM is the LanC homologue; the other domain may perform the same or similar function as LanB. Although there is now indirect evidence that PepC is involved in thioether bond formation, it remains to be determined experimentally whether these encoded proteins are indeed the modifying enzymes and what the underlying catalytic mechanism could be. Further modifications like D-alanine formation and N- or C-terminal modification only occur in specific lantibiotics and presumably require other enzymes encoded by genes found only in the corresponding gene cluster (e.g. *epiD* and several *las* genes).

Secretion and cleavage of the leader peptide are the final steps in activation of the lantibiotic. The order in which these two steps occur appears to depend on the type and location of the leader peptidase and translo-

```
Sensor kinase
NisK    (233) -LSHDVKTPLTVLKGNIELL- (88) -LSRALINIFVNA- (23) -FEIWNNGHPF- (24) -GIGLSFA- (29)   447
SpaK    (245) -LAHEIKIPITIIKGNAELL- (88) -LHRALLNILTNA- (22) -FFVKDTGNGF- ?                    >387 ?
PhoR    (355) -VSHELKTPITSIKGFTETL- (91) -LKQVFLNLVNNA- (22) -IEVADSGIGI- (26) -GLGLAIV- (35)   579
EnvZ    (240) -VSHDLRTPLTRIRLATEMM- (77) -IKRAVANMVVNA- (20) -FQVEDDGPGI- (24) -GLGLAIV- (41)   450

consensus       .sH..ktP.T.ikg..E..         l.ra..N...NA         f.v.d.G.g.         G.GL...
                  *                                   *              * * *          * *


Response regulator
NisR          VVYKILIVDDD- (36) -DLILLDIMMS- (17) -PIIFVSAKDTE- (9) -GGDDYITKPF-
SpaR           MAKILAVDDE- (35) -DLILLDVMMP- (17) -PILFLTAKTEE- (9) -GGDDYITKPF-
PhoP          MNKKILVVDDE- (36) -DLIVLDVMLP- (18) -PILMLTAKDEE- (9) -GADDYMTKPF-
OmpR        MQENYKILVVDDD- (36) -HLMVLDLMLP- (18) -PIIMVTAKGEE- (9) -GADDYIPKPF-

consensus       ..KIL.VDD.          dLi.LD.M.p          PI...tAK.eE          G.DDYitKPF
                          *                 *                                          *
                                                                        residues
NisR      - (53) -LTCREYDILELLSQR- (44) -IKTVRGLGY- (4)       229
SpaR      - (47) -LTKNEYKICEFLAQH- (44) -IKTVWGVGY- (3)       220
PhoP      - (56) -LTPKEFELLLYLGRH- (47) -IKTIRGLGY- (9)       241
OmpR      - (53) -LTSGEFAVLKALVSH- (46) -IQTVWGLGY- (9)       239

consensus         LT..E...l..L..h         IkTv.GlGY
```

Fig. 12. Strongest homology regions of two-component regulators: sensor kinase "LanK" and response regulator "LanR". PhoR/PhoP, *Bacillus subtilis* sensor/regulator; EnvZ/OmpR, *Escherichia coli* sensor/regulator; *, essential residues. Other details as in Fig.2.

cator. The ABC exporter LanT is encoded in each gene cluster and it is therefore predicted to be involved in translocation of lantibiotics. The subtilisin-like leader peptidases PepP, ElkP and LasP are predicted to remain intracellular, due to the lack of a signal sequence; NisP, EpiP and CylP are predicted to function extracellularly and for these three enzymes this has been confirmed experimentally. A novel protease for leader peptide cleavage of cytolysin and lacticin 481 precursors is postulated to be a separate intracellular domain of CylT and LctT. These various functions may be performed by a biosynthetic, membrane-bound complex of proteins consisting of LanT, LanB/LanC (or LanM) and in some cases LanP. The LanP could be attached at the internal side of such a complex (i.e. PepP, LasP, ElkP), while NisP and possibly EpiP and CylP may be attached to the extracellular side.

Various factors are assumed to contribute to self-protection or immunity of the producing strain against its own lantibiotic, and the mechanisms may differ in each organism. One of these factors appears to be an extracellular, membrane-bound LanI protein in the case of protection against nisin, subtilin and Pep5; these proteins do not show sequence similarity and hence may function quite differently. Another ABC exporter system, consisting of LanE, LanF and LanG proteins, has only recently been implicated in self-protection against nisin, subtilin and epidermin. No information is available on self-protection against lacticin 481, lactocin S and cytolysin, since neither this LanFEG system nor LanI appear to be encoded in their gene clusters. In this respect it can not be excluded that the N-terminal domain of the unique LanM protein in these three systems may play a role in self-protection.

Finally, information on regulation of expression of these gene clusters is very limited. A well-known two-component regulatory system LanR/LanK is only encoded in the nisin and subtilin gene clusters. LanK is presumably a membrane-spanning histidine kinase that needs to interact with an extracellular signal, which in the case of nisin is the lantibiotic itself (Kuipers et al., 1995), in order to activate the intracellular LanR response regulator that triggers gene expression.

This compilation of lantibiotic gene clusters and encoded proteins provides a comprehensive database which can be used for a rapid screening of newly determined nucleotide and amino acid sequences. The comparison of sequences has led to predictions of the function and cellular location of the various encoded proteins and their domains. Consensus sequences and conserved residues have been identified which are pre-

dicted to be essential for structure and/or function of these proteins. These predictions provide a useful and more focussed starting-point for genetic and protein engineering studies, but also for biochemical studies to unravel the complex mechanisms of biosynthesis, secretion, activation and immunity of individual lantibiotics.

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